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The environmental tolerances and metabolic physiology of sablefish (*Anoplopoma fimbria*)[☆]

Robine H.J. Leeuwis^{*}, Gordon W. Nash, Rebecca M. Sandrelli, Fábio S. Zanuzzo, Anthony K. Gamperl

Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL A1C 5S7, Canada

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ABSTRACT

Given the potential impacts of global warming, such as increases in temperature and the frequency/severity of hypoxia in marine ecosystems, it is important to study the impacts of these environmental challenges on sea-cage reared aquaculture species. This study focuses on the sablefish (*Anoplopoma fimbria*), an emerging aquaculture species that has a unique ecology in the wild. For instance, adults inhabit oxygen minimum zones and cool waters at depths up to 1500 m. Using Atlantic salmon (*Salmo salar*) (~1132 g adults) as a comparative species, we used intermittent-flow respirometry to characterize the tolerance and metabolic response of sablefish (~10 g juveniles and ~675 g adults) to acute increases in temperature (2 °C h⁻¹) and decreases in oxygen level (~10% air saturation h⁻¹). Adult sablefish were much more hypoxia tolerant than adult salmon [O₂ level at loss of equilibrium ~5.4% vs. ~24.2% air saturation, respectively]. In addition, sablefish could withstand upper temperatures only slightly lower than salmon [critical thermal maximum (CT_{max}) ~24.9 °C vs. ~26.2 °C, respectively]. Sablefish juveniles were both less hypoxia and thermally tolerant than adults [critical O₂ tension ~18.9% vs. ~15.8% air saturation; CT_{max} ~22.7 vs. ~24.9 °C, respectively]. Interestingly, many of these differences in environmental tolerance could not be explained by differences in metabolic parameters (aerobic scope or routine metabolic rate). Our findings show that sablefish are tolerant of high temperatures, and very tolerant of hypoxia, traits that are advantageous for an aquaculture species in the era of climate change.

1. Introduction

Fish inhabit a wide array of ecosystems, in which environmental conditions such as oxygen level and temperature vary greatly. However, these environmental conditions are getting closer to, or are already exceeding, the tolerance limits of numerous fish species and/or populations (Booth et al., 2017; Cheung et al., 2009; Comte and Olden, 2017). This is because freshwater and marine habitats (especially coastal areas where the majority of aquaculture activities takes place) are experiencing elevated temperatures (Doney et al., 2011; IPCC, 2013; Masson and Cummins, 2007; Wernberg et al., 2011) and hypoxia; the latter due to eutrophication and global warming associated with anthropogenic activities (Breitburg et al., 2018; Claret et al., 2018; Diaz and Breitburg, 2009; Diaz and Rosenberg, 2008). What mechanisms determine the tolerance of fish to these environmental conditions, and what processes will shape a species' success and survival (and ultimately its future ecological niche and distribution) are the focus of a major scientific effort (e.g., see McKenzie et al., 2016).

The sablefish (*Anoplopoma fimbria*) is an interesting model for studying the environmental tolerance of marine fishes and the underlying determinants. It is widely distributed along the North Pacific, and while juveniles live in-shore, the adults inhabit cold and deep waters (up to 1500 m) that are often severely hypoxic (Mason et al., 1983). For example, adult sablefish are known to inhabit the oxygen minimum zone off the California coast where oxygen levels range from 0.34 to 0.80 mg O₂ L⁻¹ (Moser et al., 1994). Furthermore, this species supports significant capture fisheries and is an emerging aquaculture species. With regards to aquaculture, although there is currently only one company growing sablefish commercially (Golden Eagle Sable Fish, Salt Spring Island, B.C., Canada), this species holds great promise. The sablefish adapts well to aquaculture rearing (Gores and Prentice, 1984), is a good candidate as a fed trophic level for integrated multi-trophic aquaculture (Reid et al., 2017), has a very high market value (DFO, 2018) and there are increasing consumer demands from emerging markets (Sonu, 2014). Nevertheless, very limited information is available on the sablefish's metabolism and environmental tolerances (see

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^{*} Corresponding author.

E-mail address: rhjleeuwis@mun.ca (R.H.J. Leeuwis).

Sullivan and Smith Jr, 1982), and metrics such as its critical thermal maximum (CT_{max}), critical O_2 tension (P_{crit}) and O_2 level at loss of equilibrium have not been determined.

The concept of Oxygen and Capacity Limited Thermal Tolerance (OCLTT) [for reviews, see Pörtner, 2010; Pörtner et al., 2017] hypothesizes that aerobic scope (AS) [the difference between standard and maximum metabolic rate (SMR, MMR)] is the primary factor that determines the upper thermal tolerance of aquatic organisms, including fishes. The OCLTT concept is supported with considerable evidence in numerous teleost species (Anttila et al., 2013; Clark et al., 2011; Eliason et al., 2011; Farrell, 2009; Farrell et al., 2008; Kelly et al., 2014; Pörtner and Knust, 2007; Sylvestre et al., 2007). However, there are also experimental findings (especially those that have measured acute thermal tolerance; CT_{max}) that contradict this concept (Brijs et al., 2015; Ekström et al., 2016; Ern et al., 2016; Gräns et al., 2014; Lefevre, 2016; Norin et al., 2013; Wang et al., 2014). Thus, the validity and relevance of the OCLTT to fish biology is currently being hotly debated (Clark et al., 2013; Pörtner et al., 2017; Jutfelt et al., 2018; Pörtner et al., 2018).

In addition, it has been proposed that the acute hypoxia tolerance of fishes is dependent on their metabolic rate (Claireaux and Chabot, 2016; Barnes et al., 2011; Farrell and Richards, 2009). In this case, routine metabolic rate (RMR) is thought to be an important determinant of acute hypoxia tolerance, as fish with relatively low RMR have less difficulty in meeting their metabolic demands when exposed to a limited environmental O_2 supply. This concept is also supported by experimental findings in various fish species (Dan et al., 2014; Barnes et al., 2011; Fu et al., 2011; Mandic et al., 2009; McKenzie et al., 2008), although it has not been explored as much as the OCLTT concept. Parameters commonly used for comparing hypoxia tolerance are the P_{crit} (defined here as the O_2 level at which the RMR starts to rapidly decline due to a switch from O_2 regulation to conformity) and the O_2 level at loss of equilibrium (LOE). Although, Wood (2018) recently questioned whether P_{crit} provides a valuable metric with regards to a fish's hypoxia tolerance and suggested alternative approaches.

In this study, we used intermittent-flow respirometry to characterize the metabolic response and tolerance of 10–12 °C acclimated sablefish (~10 g juveniles and ~675 g adults) to acute incremental increases in temperature (2 °C h⁻¹) or stepwise decreases in the O_2 level (~10% air saturation h⁻¹). Furthermore, experiments were concurrently performed on Atlantic salmon (*Salmo salar*) adults (~1130 g) to allow for a direct comparison of metabolic parameters and tolerance limits with those for the sablefish. Such parallel studies need to be performed if meaningful insights into inter-specific differences in tolerance limits, and their determinants, are to be obtained. Disparities in methodologies (e.g., acclimation temperatures/conditions, experimental conditions, rate of temperature increase or O_2 decrease) can significantly impact the results of such experiments. We chose the Atlantic salmon as a comparative species because it is a eurythermal fish whose environmental tolerances have been reported previously (e.g., Anttila et al., 2013; Anttila et al., 2015; Hvas et al., 2017), and it is an important aquaculture species world-wide that is experiencing elevated temperatures and hypoxic conditions at cage-sites (Burt et al., 2012; Oldham et al., 2017; Oppedal et al., 2011; Stehfest et al., 2017). Based on the ecology/life history of the sablefish, we hypothesized that this species would be less thermally tolerant than the Atlantic salmon, but more hypoxia tolerant. Further, we expected that differences between life-stages and species in upper temperature and hypoxia tolerance would be related to variations in metabolic parameters.

2. Materials and methods

2.1. Animals and holding conditions

Juvenile sablefish (~0.5 g) were acquired from the NOAA Manchester Research Station (Port Orchard, Washington, USA) and

initially kept in 500 L tanks supplied with seawater at 10–11 °C and with 24 h light. These fish were eventually transferred to 3000 L tanks at the same temperature, but on a 12 light: 12 h dark photoperiod. Atlantic salmon smolts were obtained from Northern Harvest Smolt Ltd. (Stephenville, Newfoundland, Canada) and held in 3000 L seawater tanks at 12 °C and on a 12 h light: 12 h dark photoperiod. Sablefish and Atlantic salmon were fed commercial cod/haddock and salmonid diets (Skretting), respectively, at a ration of 2.5–5.0% (juveniles) and 0.75–1.00% (adults) of body mass day⁻¹. However, all fish were fasted for one day prior to experiments. All experimental procedures were performed at the Department of Ocean Sciences of Memorial University of Newfoundland (MUN), St. John's, Canada, and were approved by MUN's Animal Care Committee (protocol number 16–92-KG).

2.2. Experiments with juvenile sablefish

The tolerance limits and metabolic physiology of juvenile sablefish were investigated in two separate experiments: an acute upper thermal (CT_{max}) challenge, and an acute hypoxic (P_{crit}) challenge. Each experiment was 2 days long, included 4 fish, and was repeated 4 times (total $n = 16$ fish per experiment). On the first day, 4 fish were anaesthetized with 0.15 g L⁻¹ tricaine methanesulphonate (MS-222; Aqualife TMS, Syndel Laboratories Ltd) and body mass was recorded. The fish were then placed into 400 mL cylindrical glass respirometers (9.2 cm in diameter × 6.1 cm high), and allowed to settle for ≥5 h. Mass-specific O_2 consumption ($\dot{M}O_2$) (mg O_2 kg⁻¹ h⁻¹) was continuously measured overnight using 15 min cycles, each composed of “flushing” (8 min), “wait” (2 min) and “recirculation” periods (5 min). Typically, $\dot{M}O_2$ decreased quickly in the first 2.5 h and remained stable overnight. These overnight measurements were used to determine standard metabolic rate (SMR) and routine metabolic rate (RMR), which were calculated as the mean of the lowest 10% of all recorded values, and the mean of all values recorded during the last 4 h of the night, respectively. The water in the respirometers was constantly replaced by aerated water from an ambient tank, and kept at 12 °C and 90–100% air saturation unless mentioned otherwise.

On the following day, in the case of the acute upper thermal challenge, $\dot{M}O_2$ was continuously measured while the temperature of the water was increased by 2 °C h⁻¹. The CT_{max} was determined as the temperature at which the fish lost equilibrium. In the case of the acute hypoxia challenge, $\dot{M}O_2$ was measured at each of the following O_2 levels: 100%, 60%, 50%, 40%, 30%, 25%, 20% and 15% air saturation. The O_2 level was decreased to these levels in a step-wise manner every 60 min until the O_2 level at which the fish lost equilibrium (LOE) was reached. To regulate the O_2 level in the respirometers and surrounding water bath, we used the same custom-built solenoid valve system that has been described by Motyka et al. (2017).

In both experiments, fish were recovered after LOE by rapidly cooling the water to 12 °C or by increasing the O_2 level to 100% air saturation. Metabolic rate was measured with intermittent-flow respirometry, using a computer running AutoResp software 2.2.2 (Loligo Systems, Viborg, Denmark) and the same equipment and procedures as described by Zanuzzo et al. (2015) with the following slight modifications. Because $\dot{M}O_2$ varied strongly with temperature and O_2 level, the durations of the “flushing” and “recirculation” periods were adjusted throughout the CT_{max} test. In this way, a $R^2 > 0.90$ for each measurement was achieved, while ensuring that the O_2 level in the chambers did not drop below 90% air saturation.

2.3. Experiments with adult sablefish and Atlantic salmon

Similar to the experiments with juveniles, the tolerance limits and metabolic response of adult sablefish and Atlantic salmon were investigated in two experiments; either an acute upper thermal or a hypoxic challenge. Each experiment was 2–3 days long and was repeated 9–13 times for each species. On the first day, one fish from each species

Table 1

Body and cardiac morphometrics for adult sablefish (*A. fimbria*) and Atlantic salmon (*S. salar*).

	Sablefish	Atlantic Salmon
Body morphometrics	<i>n</i> = 22	<i>n</i> = 20
Body mass (g)	675 ± 19	1132 ± 54*
Length (cm)	39.1 ± 0.4	45.0 ± 0.6*
Condition factor	1.11 ± 0.02	1.19 ± 0.02*
Cardiac morphometrics	<i>n</i> = 21	<i>n</i> = 19
RVM ^a (%)	0.086 ± 0.002	0.066 ± 0.002*
RBM ^b (%)	0.021 ± 0.001	0.019 ± 0.001
BM/VM ^c	0.243 ± 0.008	0.289 ± 0.008*

Values are means ± S.E.M.

* A significant difference ($P < .05$) between adult sablefish and Atlantic salmon.

^a Relative ventricular mass.

^b Relative bulbar mass.

^c Ratio between bulbar mass and ventricular mass.

was anaesthetized with 0.1 g L⁻¹ MS-222, and body mass and fork length were recorded. The fish were then placed into cylindrical plexiglass respirometers that were submerged in a “water table”, and allowed to settle for ≥24 h. Because the two species in this study had significantly different body masses (Table 1), two different sizes of respirometer were used (14.1 L respirometers for sablefish [17.2 cm in diameter × 61.0 cm long] and 19.8 L respirometers for salmon [20.3 cm in diameter × 61.0 cm long]). The position of the respirometers (*i.e.*, at the front or back of the water table) for each experiment was randomized. The O₂ level in the water was regulated by a control system (OXY-REG, Loligo Systems) that monitored the O₂ level with a galvanic O₂ probe (MINI-DO), and that made adjustments to the water's O₂ level by either releasing O₂ or N₂ into the seawater reservoir that supplied the water table.

After the initial 24 h period, in the case of the acute upper thermal challenge, $\dot{M}O_2$ was continuously measured for 24 h to determine SMR and RMR, followed by a thermal challenge on day 3 (using the same procedure as described for the juveniles). For the overnight $\dot{M}O_2$ measurements, 20 min cycles were used, whereby the length of the “flushing”, “wait” and “recirculation” periods varied slightly (due to variations in body size and $\dot{M}O_2$) but were approximately 12 min, 2–3 min, and 5–6 min. In the case of the acute hypoxia challenge, $\dot{M}O_2$ was measured twice at each of the following O₂ levels: 90–100%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% and 5% air saturation. The O₂ level was decreased by 10% h⁻¹ until 10% air saturation was reached, after which the O₂ level was lowered to 5% over an hour and maintained at this level of air saturation till LOE occurred. Fish were acclimated to the new O₂ level for a minimum of 10 min before $\dot{M}O_2$ measurements were made. The O₂ level at LOE was recorded, as well as the time till LOE at 5% air saturation (in sablefish). If possible, more than two $\dot{M}O_2$ measurements were made at 5% air saturation. $\dot{M}O_2$ measurements were made using the same equipment and control systems as used for the juvenile sablefish.

In both experiments, fish were euthanized in 0.2 g L⁻¹ MS-222 after LOE occurred. Because one fish from each species were tested in parallel, and CT_{max} and the O₂ level at LOE varied between individuals and species (Table 2), the fish that reached its tolerance limit first was euthanized immediately by injecting 0.2 g L⁻¹ MS-222 into its respirometer while in the “recirculation” mode. This allowed us to continue measurements on the remaining fish while ensuring that policies for the ethical treatment of animals were followed. Once the remaining fish reached its LOE, it was immediately taken out of the respirometer and euthanized. Finally, the ventricular and bulbar masses of each fish

were measured, and expressed as a percentage of body mass [relative ventricular mass (RVM) and bulbar mass (RBM), respectively].

2.4. Data analyses

To account for the well-known effect of body size on metabolic rate, all $\dot{M}O_2$ values (mg O₂ kg⁻¹ h⁻¹) were adjusted using an exponent of 0.80 to obtain allometrically scaled values (*i.e.*, in mg O₂ kg^{-0.80} h⁻¹). We are aware that scaling exponents can vary depending on many factors such as species, life style, hierarchical level and the metabolic rate parameter being measured (*i.e.*, SMR vs. RMR. vs. MMR vs. AS), however we chose the scaling exponent of 0.80 because this value agrees with several studies that report SMR and RMR data on > 90 teleost species (Clarke and Johnston, 1999; Killen et al., 2010; Killen et al., 2007; Norin and Gamperl, 2018). Further: 1) to our knowledge no scaling exponent for Atlantic salmon metabolic parameters has been reported, and this value is in the middle of the range of scaling factors that have been reported for salmonids (sockeye salmon: 0.87–0.88; steelhead trout: 0.73) (Brett and Glass, 1973; Trudel and Welch, 2005); and 2) although Sullivan and Smith Jr (1982) reported an allometric scaling exponent of 0.72 for sablefish RMR, there were several serious shortcomings in their analysis, and we were not confident using their data or the exponents that could be calculated in this study based solely on two size classes of fish (which ranged from 0.76 for SMR to 0.80 for AS_T).

The P_{crit} was determined for each individual fish using a method similar to Monteiro et al. (2013) by: 1) calculating the mean of the RMR measurements during the range of regulation; 2) calculating the linear regression for the $\dot{M}O_2$ values that were dependent on water O₂ content (*i.e.*, after the fish became an oxyconformer); and 3) calculating the O₂ level at the intersection between the RMR and the calculated linear regression (see Fig. S1). We used RMR rather than the fish's SMR to determine P_{crit} because this is the most common approach in studies of P_{crit}; it provides a P_{crit} value that is more relevant than a P_{crit} based on the SMR (*e.g.*, see Rogers et al., 2016; Wood, 2018), and this method has been used in aquaculture-based studies where it is referred to as the limiting oxygen saturation (LOS, Barnes et al., 2011; Remen et al., 2013). Further, fish likely exhibit a routine level of $\dot{M}O_2$ the majority of the time, whereas even under experimental conditions that restrict the fish's movement and remove stimulation, $\dot{M}O_2$ values representative of SMR are infrequently measured.

Aerobic metabolic scope was calculated by subtracting the SMR for each fish at its acclimation temperature from its maximum metabolic rate (MMR) during the incremental temperature increase; the latter value determined as the highest $\dot{M}O_2$ value measured during the thermal challenge (irrespective of the temperature at which it was measured). We recognize that this way of measuring aerobic scope (which we will define as AS_T) is different from the traditional method where SMR and MMR are measured at the same temperature, and MMR is determined by exercising fish to their critical swimming speed (U_{crit}) (*e.g.* see Brett, 1964). However, in an acute upper thermal challenge, the fish's ability to elevate its metabolism as temperature increases is likely to be the most relevant parameter to look at with respect to how “metabolic capacity” is related to a fish's thermal tolerance. In addition, previous studies (see Gollock et al., 2006 vs. Petersen and Gamperl, 2010; Powell and Gamperl, 2016) have shown that AS_T in adult cod is within 10–20% of their aerobic scope as measured by performing a U_{crit} test (*i.e.*, their AS). These two measures are also more comparable/similar than when MMR is measured by chasing fish to exhaustion (another commonly used method of determining AS) vs. performing a U_{crit} test (Reidy et al., 1995; Norin and Clark, 2016). Finally, it has recently been suggested that the relationship between acclimation temperature and AS is similar when obtained by giving the fish a chase or U_{crit} test vs. giving them an incremental temperature increase at each acclimation temperature and measuring their thermal metabolic scope (TMS;

Table 2Metabolic parameters, and acute upper thermal and hypoxia tolerance, in juvenile and adult sablefish (*A. fimbria*) and adult Atlantic salmon (*S. salar*).

	Sablefish		Atlantic Salmon
	Juvenile (~10 g)	Adult (~675 g)	Adult (~1132 g)
Metabolic parameters	<i>n</i> = 32	<i>n</i> = 13	<i>n</i> = 11
SMR (mg O ₂ kg ^{-0.80} h ⁻¹) ^a	67.4 ± 2.5 ⁺	57.6 ± 3.4	75.3 ± 3.7 [*]
RMR (mg O ₂ kg ^{-0.80} h ⁻¹) ^b	94.5 ± 3.7 ⁺	81.0 ± 4.6	104.4 ± 3.8 [*]
Acute upper thermal tolerance	<i>n</i> = 14	<i>n</i> = 10	<i>n</i> = 10
MMR (mg O ₂ kg ^{-0.80} h ⁻¹) ^c	260.3 ± 15.4	234.3 ± 5.9	265.0 ± 11.5 [*]
AS _T (mg O ₂ kg ^{-0.80} h ⁻¹) ^d	183.2 ± 15.8	176.9 ± 7.0	188.5 ± 11.3
T _{crit} (°C) ^e	21.8 ± 0.3	22.7 ± 0.4	23.7 ± 0.5
CT _{max} (°C) ^f	22.7 ± 0.3 ⁺	24.9 ± 0.2	26.2 ± 0.5 [*]
Acute hypoxia tolerance	<i>n</i> = 13	<i>n</i> = 9	<i>n</i> = 9
RMR during regulation (mg O ₂ kg ^{-0.80} h ⁻¹)	75.5 ± 2.2	64.9 ± 7.5	79.7 ± 6.6
P _{crit} (% air sat) ^g	18.9 ± 0.4 (10) ⁺	15.8 ± 1.5 (8)	N/A
O ₂ level at LOE ^h (% air sat)	8.3 ± 0.4 ⁺	5.4 ± 0.5	24.2 ± 1.8 [*]
Time till LOE at 5% air sat (min) ⁱ	N/A	45 ± 11 (12–106)	N/A

Values are means ± S.E.M.

⁺ A significant difference (*P* < .05) between juvenile and adult sablefish.^{*} A significant difference (*P* < .05) between adult sablefish and Atlantic salmon.^a Standard metabolic rate.^b Routine metabolic rate.^c Maximum metabolic rate; measured as the highest metabolic rate for each fish independent of the temperature at which it was recorded.^d Aerobic scope, calculated as the difference between SMR measured at the fish's acclimation temperature and the MMR recorded for each fish.^e Critical temperature, the temperature at which the highest metabolic rate was measured, before fish started to lose equilibrium.^f Critical thermal maximum.^g Critical oxygen level, P_{crit}. P_{crit} could not be identified for 3 juvenile and 1 adult sablefish, thus, sample size was reduced to *n* = 10 and *n* = 8, respectively. P_{crit} could also not be identified with confidence for any Atlantic salmon individuals tested.^h Loss of equilibrium.ⁱ Time till fish lost equilibrium while kept at an O₂ level of 5% air saturation. For this parameter, the range of values recorded is shown in the brackets.

Paschke et al., 2018). Thus, we feel that AS_T is a valuable metric for examining how the capacity of fish to elevate their metabolic rate is related to their acute thermal tolerance, and that it provides comparable information to that obtained by calculating AS based on exhaustive exercise protocols.

In this study, the critical temperature (T_{crit}) was also estimated for each group (i.e., juvenile sablefish, adult sablefish, and adult salmon) and defined as the temperature where MMR was reached before each fish lost equilibrium.

2.5. Statistical analyses

Outliers were identified as values with a standardized residual of < -3 or > 3 and omitted from the dataset. Assumptions of normality and homoscedasticity were subsequently tested using the Shapiro-Wilk and Fligner-Killeen tests, respectively. Differences in morphometric, metabolic, and tolerance parameters (Tables 1 and 2) between life-stages (juvenile vs. adult sablefish) and species (sablefish vs. salmon adults) were identified using student-*t*-tests, or Mann-Witney *U* tests if the above assumptions failed. The effect of temperature or O₂ level on $\dot{M}O_2$ (Fig. 1) within each group (i.e., juvenile sablefish, adult sablefish, adult salmon) was determined using one-way ANOVA's, and data was transformed in the case of non-normality and heterogeneity. If the effect of temperature or O₂ level was significant, differences between the starting temperature (12 °C) and higher temperatures, or between the starting O₂ level (90–100% air saturation) and lower O₂ levels, were identified using Dunnett's *post-hoc* comparisons. All statistical analyses were performed using R (version 3.4.0) and RStudio software, and with *P* < .05 as the level of significance. All values reported in the text, figures and tables are means ± 1 standard error of the mean (S.E.M.).

3. Results

$\dot{M}O_2$ data recorded during the acute thermal and hypoxia challenges for 5 juvenile and 3 adult sablefish, and 1 adult Atlantic salmon, were not used in the final analysis. These fish struggled, which resulted in 2-to-3-fold elevations in $\dot{M}O_2$ compared to the previous $\dot{M}O_2$ measurement, even though the temperature or O₂ level had changed by only 1 °C or 10% air saturation. However, the body and cardiac morphometrics, SMR and RMR for these fish were included in the data set.

3.1. Body and cardiac morphometrics

The adult sablefish and Atlantic salmon used in our experiments differed significantly in body mass (*P* < .0001), length (*P* < .0001) and condition factor (*P* = .006); all these parameters being higher for the salmon. In contrast, the sablefish had a significantly higher RVM than the Atlantic salmon (0.086 ± 0.002% vs. 0.066 ± 0.002%, respectively; *P* < .0001) (Table 1). While no difference in RBM existed (~0.020% for both species), the ratio between bulbar and ventricular masses was lower in sablefish as compared to the salmon (0.243 ± 0.008% vs. 0.289 ± 0.008%; *P* = .0002).

3.2. Standard and routine metabolic rates

The SMR (57.6 ± 3.4 mg O₂ kg^{-0.80} h⁻¹) and RMR (81.0 ± 4.6 mg O₂ kg^{-0.80} h⁻¹) of adult sablefish were significantly lower as compared to both the juvenile sablefish (by ~14–15%) and the adult salmon (by ~22–24%) (Table 2).

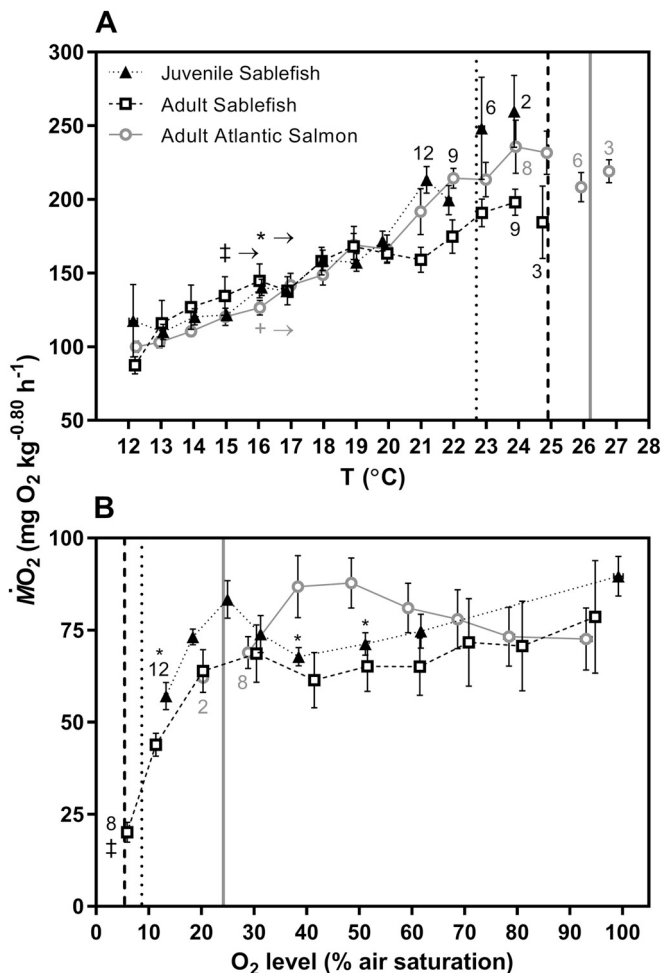


Fig. 1. Mass-specific O_2 consumption ($\dot{M}O_2$, $\text{mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$) of juvenile and adult sablefish (A. fimbria) and adult Atlantic salmon (S. salar) measured using intermittent-flow respirometry, as affected by increases in water temperature ($^{\circ}\text{C}$) (A) and reductions in water O_2 level (% air saturation) (B). Vertical lines indicate the average CT_{max} (A) or O_2 level at loss of equilibrium (LOE) (B), and numbers indicate reduced sample sizes due to LOE. When the sample size became < 8 fish, data points were no longer connected with a line. Data are means $\pm$ S.E.M. See Table 2 for sample sizes, and values for SMR, RMR, MMR, CT_{max} , T_{crit} , P_{crit} , and O_2 level at LOE based on these data. Significant differences ($P < .05$) between the $\dot{M}O_2$ at the starting temperature (12°C) vs. higher temperatures, or at the starting O_2 level (90–100% air saturation) vs. lower O_2 levels, are indicated with * for juvenile sablefish, + for adult sablefish, and + for Atlantic salmon. The horizontal arrows (A) indicate that there is a significant difference at all following temperatures.

3.3. Acute upper thermal tolerance and metabolic parameters

The $\dot{M}O_2$ of all groups was significantly higher by 15–16 $^{\circ}\text{C}$ as compared to that measured at the fish's acclimation temperature (12°C). The acute upper thermal tolerance (CT_{max}) of the adult sablefish was approx. 2.2°C higher than that of juveniles ($24.9 \pm 0.2^{\circ}\text{C}$ vs. 22.7 ± 0.3 , respectively; $P < .0001$), but approx. 1.3°C lower than that of the Atlantic salmon ($26.2 \pm 0.5^{\circ}\text{C}$, respectively; $P = .018$) (Table 2, Fig. 1A), and this pattern was also reflected in their T_{crit} values (juvenile sablefish: $21.8 \pm 0.3^{\circ}\text{C}$ vs. adult sablefish: 22.7 ± 0.4 vs. adult salmon: $23.7 \pm 0.5^{\circ}\text{C}$). Despite these differences in CT_{max} , AS_T values for all three groups were very similar (juvenile sablefish, $183.2 \pm 15.8 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$; adult sablefish, $176.9 \pm 7.0 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$; adult salmon, $188.5 \pm 11.3 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$) (Table 2). This was largely because life-stage or species differences in SMR offset any differences in maximum metabolic rate (MMR); the MMR of both

the juvenile sablefish and adult salmon being ~ 11 – 13% higher than what was recorded for the adult sablefish.

3.4. Acute hypoxia tolerance and metabolic parameters

The acute hypoxia tolerance of adult sablefish was greater than that of juveniles as indicated by the significantly lower P_{crit} values ($15.8 \pm 1.5\%$ vs. $18.9 \pm 0.4\%$ air saturation, respectively; $P = .037$) and the significantly lower O_2 level at LOE ($5.4 \pm 0.5\%$ vs. $8.3 \pm 0.4\%$ air saturation, respectively; $P = .0002$) (Table 2, Fig. 1B). While the mean values for RMR during the period of O_2 regulation were similar for the two groups (64.9 ± 7.5 vs. $75.5 \pm 2.2 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$, respectively; $P = .229$), there was a difference in the nature of the $\dot{M}O_2$ response to decreases in the water O_2 level (Fig. 1B). While the $\dot{M}O_2$ of adult sablefish changed very little between 40% and 20% air saturation, $\dot{M}O_2$ in the juveniles at 25% air saturation increased by approx. 23% (compared to that at 40% air saturation) before declining. This was associated with an increase in swimming activity of the juvenile sablefish.

P_{crit} could not be identified with confidence for any of the Atlantic salmon tested using our protocol, and could therefore, not be compared with that of sablefish. This was because none of the Atlantic salmon in this study showed a clear reduction in $\dot{M}O_2$ at low O_2 levels (O_2 conformity) and instead, maintained a relatively stable $\dot{M}O_2$ until LOE (see Fig. S1). Indeed, $\dot{M}O_2$ did not significantly decline with the lowering of water O_2 levels in Atlantic salmon (Fig. 1B). The O_2 level at LOE for the sablefish was much lower than that of the Atlantic salmon ($5.4 \pm 0.5\%$ vs. $24.2 \pm 1.8\%$ air saturation, respectively; $P = .0004$) (Table 2, Fig. 1B). Furthermore, sablefish adults were able to maintain equilibrium at 5% air saturation for a considerable amount of time (on average 45 min; range 12–106 min).

4. Discussion

In this study, we showed that the sablefish is quite tolerant of elevated temperatures and highly hypoxia tolerant. These are advantageous traits in aquaculture, given that marine ecosystems are projected to experience higher temperatures (IPCC, 2013) and more frequent and severe hypoxic conditions as a result of climate change (Breitburg et al., 2018; Diaz and Breitburg, 2009; Diaz and Rosenberg, 2008). In addition, aquaculture cage-sites along the British Columbia (Canada) coast are currently experiencing periods of severe hypoxia associated with frequent changes in ocean currents that result in the upwelling of large pockets of deep water that are low in oxygen (see <http://marineharvest.ca/about/news-and-media/container2015/15-december-2015/>).

4.1. Routine metabolic rate

Our measurements for RMR in $\sim 0.68 \text{ kg}$ sablefish at 12°C (~ 65 – $81 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$; Table 2) are within the range of values reported by Sullivan and Smith Jr (1982) for $\geq 0.7 \text{ kg}$ sablefish ($\sim 75 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$). Further, the RMR value we report for the Atlantic salmon ($\sim 1.1 \text{ kg}$; ~ 80 – $104 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$) at 12°C is comparable to that measured in $\geq 0.15 \text{ kg}$ post-smolt salmon at 10 – 14°C by Deitch et al. (2006) ($\sim 63 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$), Lucas (1994) ($\sim 84 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$) and Barnes et al. (2011) ($\sim 101 \text{ mg O}_2 \text{ kg}^{-0.80} \text{ h}^{-1}$).

4.2. Acute upper thermal tolerance

The CT_{max} of adult Atlantic salmon in this study ($\sim 26.2^{\circ}\text{C}$) is in line with values reported by several other authors for this species (~ 24.5 – 30°C) (Anttila et al., 2013; Anttila et al., 2015; Bowden et al., 2018), even though these studies used smaller sized fish (~ 25 – 75 g) (see below) and a much faster (and less ecologically relevant) rate of heating (0.1 – $0.3^{\circ}\text{C min}^{-1}$) (e.g., Anttila et al., 2013, 2015). The CT_{max} of adult sablefish was only $\sim 1.3^{\circ}\text{C}$ lower than that of the Atlantic

salmon, even though adult sablefish live in deep and cold waters where they would typically not encounter high temperatures (Mason et al., 1983). Further, their CT_{max} was approximately 2 °C higher than that of adult Arctic char (*Salvelinus alpinus*), which has a Holarctic distribution and a CT_{max} of ~23 °C (Penney et al., 2014). This relatively high upper thermal tolerance, while unexpected, is a beneficial trait for sablefish adults kept in sea-cages. High temperatures can be experienced during the summer months, and in the Georgia Strait (British Columbia, Canada) temperatures at all depths are increasing at a rate of ~0.024 °C per year (Masson and Cummins, 2007).

This study also shows that juvenile sablefish have a lower CT_{max} (by ~2.2 °C) than that of adult fish. This is surprising given that juveniles in the wild are more likely to encounter high temperatures in their in-shore habitat, and smaller fish generally have higher CT_{max} values [coho salmon (*Oncorhynchus kisutch*), Clark et al., 2012; leopard coral grouper (*Plectropomus leopardus*), Messmer et al., 2017; Delta smelt (*Hypomesus transpacificus*), Komoroske et al., 2014; and coral reef damselfishes, Clark et al., 2017]. The reason for this is not clear (e.g., see below), but this data suggests that juvenile sablefish may be more susceptible to high sea-cage temperatures.

In this study, variations in acute upper thermal tolerance (i.e., mean CT_{max} values ranging from ~22.7 to 26.2 °C) could not be explained by differences in AS_T . The AS_T of all three groups was approximately 180 mg O₂ kg^{-0.80} h⁻¹ (Table 2), and no significant correlations were evident within the groups between CT_{max} and AS_T (see Fig. S2). Although our experiments were primarily designed to measure the sablefish's metabolism and environmental tolerances, and were not specifically meant to systematically test the OCLTT concept, these data suggest that AS_T is not a determinant of acute thermal tolerance in these fishes (even if rates of temperature increase are ecologically relevant). This finding is supported by a large number of other studies (e.g., Brijis et al., 2015; Ekström et al., 2016; Ern et al., 2016; Gräns et al., 2014; Lefevre, 2016; Norin et al., 2013; Wang et al., 2014). While these studies do not preclude AS_T or aerobic scope (AS) from playing an important role in fish thermal tolerance when evaluated over longer time scales (i.e., days to months, etc.), they contribute to the ongoing debate about the relevance of the OCLTT concept to fish thermal biology/ecology. This concept has been challenged by a large group of scientists in the field (see Jutfelt et al., 2018), and recent data from Hvas et al. (2017) further question the relevance of the OCLTT concept. These authors showed that the CT_{max} of juvenile Atlantic salmon correlated positively with acclimation temperature, and that > 20% of fish acclimated to 23 °C died, despite AS being highest at 23 °C and similar to that measured in fish acclimated to 15 and 18 °C.

Mechanisms that determine upper thermal tolerance in fish are constantly being investigated and debated [e.g., see Jutfelt et al., 2018 vs. Pörtner et al., 2017, 2018]. It is possible, for example, that some of the differences in upper thermal tolerance (CT_{max}) between adult sablefish and salmon were determined by differences in cardiac function and the temperature sensitivity of their cardiovascular systems. In many species, CT_{max} is preceded by cardiac collapse (i.e., cardiac arrhythmias followed by a sudden fall in cardiac output) (Clark et al., 2008; Farrell, 2002; Gollock et al., 2006), and Anttila et al. (2015) recently showed that maximum heart rate and the temperature at which the heart goes arrhythmic are sensitive to acclimation temperature and highly plastic. Perhaps, cardiac collapse occurred at a lower temperature in sablefish than in Atlantic salmon, as Atlantic salmon have a coronary circulation that supplies O₂-rich blood to the ventricular myocardium, whereas the sablefish do not (pers. observation). Anttila et al. (2013) showed that the CT_{max} was positively correlated with the RVM of Atlantic salmon families, and suggested that heart size may be an important determinant of thermal tolerance. However, it is clear that heart size and blood oxygen carrying capacity did not determine the difference in CT_{max} between the adult salmon and sablefish. The RVM of sablefish was 30% larger than in the Atlantic salmon (Table 1), and haematocrit and blood [haemoglobin] in normoxic adult

sablefish acclimated to 10–11 °C are $22.5 \pm 1.0\%$ and 7.5 ± 0.3 g dL⁻¹ (Leeuwis et al., unpubl.). Both these latter values are lower than for the Atlantic salmon stocks that we use for experiments at Memorial University (Deitch et al., 2006; Harter et al., submitted).

4.3. Acute hypoxia tolerance

Our findings clearly show that the sablefish is a highly hypoxia tolerant species, and are in agreement with Sullivan and Smith Jr (1982). These authors showed that the RMR of unfed sablefish did not fall appreciably between 8 and 1 mg L⁻¹ O₂ (~15% saturation). This conclusion is also supported when our data are compared with that collected for other fish species. For example, the P_{crit} for juvenile and adult sablefish (~18.9% and ~15.8% air saturation, respectively) is much lower than the P_{crit} of hypoxia intolerant species such as dover sole (*Solea solea*) (~49.3% air saturation) (McKenzie et al., 2008) and lumpfish (54.4% air saturation) (*Cyclopterus lumpus*) (Ern et al., 2016) at similar temperatures. Moreover, the P_{crit} for sablefish is similar to the P_{crit} reported for relatively hypoxia tolerant species like goldfish (*Carassius auratus*) (~14.8% air saturation) (Fu et al., 2011) and several sculpins species of the Cottidae family (~16% air saturation) (Mandic et al., 2009). However, it is not as low as that of extremely hypoxia tolerant cyprinid species (~7.8% air saturation) (Fu et al., 2013). The O₂ level at LOE for sablefish juveniles and adults (~8.3% and ~5.4% air saturation, respectively) is also well below the lowest O₂ level that can be endured by Atlantic cod (~20.8% air saturation) (Petersen and Gamperl, 2010), a relatively hypoxia intolerant marine species. It is important to note here, that the O₂ level at LOE for adult sablefish is likely to be even lower than the value reported in this study. This is because our experimental set-up/equipment did not allow us to lower the O₂ level beyond 5% air saturation, and adult sablefish were able to tolerate this low O₂ level for 45 ± 11 min (Table 2).

We were not able to confidently determine the P_{crit} for adult Atlantic salmon in this study, as none of the individuals tested showed a reduction in metabolic rate at low O₂ levels that would indicate a switch from O₂ regulation to conformity (e.g., see Fig. S1). This was surprising given that other studies have successfully determined the P_{crit} for Atlantic salmon. For instance, Stevens et al. (1998) reported a P_{crit} of ~38% air saturation for ~0.04 kg pre-smolts, Barnes et al. (2011) showed that the P_{crit} for ~0.15 kg post-smolts was ~42% air saturation, and Remen et al. (2013) found that P_{crit} was ~39% air saturation for 0.3–0.6 kg fish. However, Barnes et al. (2011) also reported that only 56% of the salmon (15 out of 27) tested showed a transition from O₂ regulation to conformity, and thus, P_{crit} could not be calculated for the remaining 44% of their fish. Apparently, some Atlantic salmon individuals in their study population had the ability to reduce their metabolic rate at low O₂ levels, whereas others could not. It is possible that the 9 fish included in the present study were also unable to lower their $\dot{M}O_2$ as the water O₂ level fell. However, the methodology used in the present study might be another reason why the P_{crit} could not be clearly identified for Atlantic salmon. Stevens et al. (1998) and Barnes et al. (2011) used closed respirometry, rather than intermittent-flow respirometry as employed here. With closed respirometry, the O₂ level in the tank or respirometer is gradually/continuously lowered by the animal's respiration, and thus, allows for many metabolic rate measurements to be made at a range of O₂ levels. In contrast, in the present study, the O₂ level was decreased in a controlled, step-wise manner (by reductions of ~5–10% air saturation), and only two $\dot{M}O_2$ measurements were made per fish at each step.

With regards to the O₂ level at LOE determined for the Atlantic salmon in this study ($24.2 \pm 1.8\%$ air saturation), it is difficult to compare this data with the literature. There are very few measurements of the O₂ level at LOE in Atlantic salmon exposed to acute hypoxia. Anttila et al. (2013) showed that salmon smolts could tolerate 10% air saturation for 23 to 120 min (depending on the family), and Wood et al. (2017) reported that the O₂ at LOE for salmon was ~12.5% saturation.

However, there are several important differences between these studies and our study. Anttila et al. (2013) conducted their study at 4 °C, and both studies lowered O₂ levels at a much faster rate (Anttila et al., 2013: 1.5% min⁻¹ until 10% air saturation; Wood et al., 2017: 4–5% per minute until 45% air saturation, then 0.3% min⁻¹) than in this study. Because of the temperature dependency of $\dot{M}O_2$ and hypoxia tolerance in salmon (e.g., see Remen et al., 2013), and that rapidly decreasing water O₂ levels reduces exposure time to low oxygen conditions, these methodological differences could have allowed the salmon in the above studies to achieve lower P_{crit} and LOE values than reported here.

Overall, the high acute hypoxia tolerance of sablefish is not surprising. Adults of this species are known to colonize the oxygen minimum zone off the coast of California, where they can encounter O₂ levels of 0.34–0.80 mg L⁻¹ (Moser et al., 1994). Further, recent work in our lab shows that they can survive weeks of exposure to 20% air saturation (Leeuwis et al., unpubl.). However, it is currently not clear what mechanisms might confer their significant hypoxia tolerance. It is unlikely that this enhanced hypoxia tolerance was associated with their RVM, as a value of 0.086% is not large for fishes (Farrell and Smith, 2017). Their haematocrit (~22%; Leeuwis et al., unpubl.) is not high, and approx. midpoint between that measured in salmon (~30%) (Deitch et al., 2006; Harter et al., submitted) and inactive species such as flatfish (~15%) (Turner et al., 1983; Wood et al., 1979). The RMR of adult sablefish was only ~20% lower than that of the salmon used in these experiments (Table 2). Further, it does not appear that the sablefish's capacity for anaerobic metabolism can explain their tolerance of low oxygen conditions. Plasma lactate was barely detectable (i.e., < 0.1 mM) in normoxia-acclimated adult sablefish, those acutely exposed to 40% air saturation at 12 °C, or adult sablefish chronically acclimated to 40% and 20% air saturation at 12 °C, and only reached 5 mM when sablefish were acutely exposed to 40% air saturation and then given an acute thermal challenge to CT_{max} (Leeuwis et al., unpubl.). In contrast, plasma lactate levels are ~0.8 mM in normoxic rainbow trout at 13 °C, and at this temperature they increase rapidly after the fish are exposed to ~30% air saturation (levels 5 and 7 mM after 30 and 60 min, respectively) (Omlin and Weber, 2010).

At O₂ levels beyond the P_{crit}, the metabolic rate of both sablefish juveniles and adults decreased (by ~36% and ~74%, respectively) as compared to that measured under normoxia. One explanation for this decrease in oxygen consumption is that this species uses adaptive responses to reduce its O₂ demand to survive periods of acute hypoxia, which could include metabolic depression (Richards, 2009). It is also possible that their hypoxia tolerance is, at least in part, related to unique aspects of their red blood cell physiology. Sablefish erythrocytes lack a β -adrenergic responsive Na⁺/H⁺ exchanger (β -NHE), and the onset of the Root effect (i.e., the drop in Hb-O₂ carrying capacity due to a pH decrease) occurs at a much lower red blood cell intracellular pH (~6.9) than in other teleost fish; rainbow trout (*Oncorhynchus mykiss*) (~7.3), common carp (*Cyprinus carpio*) (~7.1) and copper rockfish (*Sebastes caurinus*) (~7.5) (Rummer et al., 2010). The copper rockfish is a species that belongs to the same taxonomic order (Scorpaeniformes) as the sablefish and inhabits a similar, but shallower habitat, where it would normally not encounter hypoxic conditions. The magnitude of the Root effect (i.e., the percentage reduction of Hb-O₂ saturation) is also considerably lower in sablefish (~35%) than in copper rockfish and rainbow trout (~50–60% reduction), and more closely resembles that of the very hypoxia tolerant common carp (~20%) (Salama and Nikinmaa, 1988). Thus, a reduced Root effect and a low onset pH might protect O₂ transport in sablefish, and this unique physiological attribute may in part explain the ability of this species to tolerate acute hypoxia and survive chronic exposure to low O₂ levels (i.e., as low as 20%; Leeuwis et al., unpubl.).

The P_{crit} and LOE of juvenile sablefish were slightly higher than measured in adult fish (~18.9% vs. ~15.6% and ~8.3% vs. ~5.4% air saturation, respectively). This difference in hypoxia tolerance is not likely due to the size difference between the two groups (Nilsson and

Östlund-Nilsson, 2008), but to the higher RMR in juvenile fish (~94.5 vs. ~81.0 mg O₂ kg^{-0.80} h⁻¹; $P = .043$) and/or the difference in behaviour as water O₂ levels were lowered. For example, the activity of juvenile sablefish increased as the water O₂ level approached 30% air saturation, whereas the adults remained calm (pers. observation); a finding supported by the increase in $\dot{M}O_2$ of the juveniles prior to reaching to their P_{crit} (Fig. 1B), and that explains why there was a strong positive relationship between P_{crit} and RMR during the O₂ regulation period in adult sablefish, but not juveniles (Fig. S3). The behavioural response to acute hypoxia involves a trade-off between increasing swimming activity to enhance the probability of finding more oxygenated water, and decreasing swimming activity to lower oxygen requirements (Killen et al., 2012), and it appears that juvenile sablefish are more proactive than their adult conspecifics. Proactive fish have been shown by van Raaij et al. (1996) to be less tolerant than fish that remain calm as water oxygen levels are lowered. This difference in behavioural response may not be unexpected, however, as juvenile sablefish live in in-shore regions that are likely to be highly oxygenated, whereas adults lead a bathydemersal existence and encounter very low oxygen levels in this habitat.

4.4. Summary

In this study, the tolerances and physiological (metabolic) responses of adult and juvenile sablefish to an acute temperature increase and acute hypoxia were examined. Based on a comparison of CT_{max}, P_{crit} and LOE values for this species with the literature, and the data we collected on Atlantic salmon, this research shows that the adult sablefish is quite tolerant of high temperatures (CT_{max} ~25 °C), and that both juvenile and adult sablefish are very tolerant of acute hypoxia (P_{crit} and LOE values of < 20% and < 9% air saturation, respectively). This data suggests that the sablefish should have no problem dealing with changes in these two environmental conditions at British Columbia (Canada) sea-cage sites; although ongoing research on the combined effects of exposure to high temperatures and hypoxia, and the physiological impacts of chronic hypoxia, will also be critical for the aquaculture industry.

Based on our inability to determine a P_{crit} value for the salmon, and the lack of a relationship between RMR and P_{crit} for juvenile sablefish (likely due to hyperactivity as the water O₂ level decreased), we are in agreement with Wood (2018) that LOE and other metrics are better suited for examining the hypoxia tolerance of fishes. This research also adds to the ongoing debate about the relevance of the OCLTT concept to fish biology/ecology/conservation [i.e., see Jutfelt et al., 2018 vs. Pörtner et al., 2017, 2018] as the tolerances of the sablefish and salmon to high temperatures in this study were not related to AS_T.

The sablefish has a unique life history and ecology, and it has already been shown that it has novel physiological attributes as compared to other teleost species (e.g., see Rummer et al., 2010). At present, however, it is unclear what specific mechanisms confer the sablefish's tolerance to the high temperatures and low oxygen conditions that were used in this study. Hopefully, recently completed experiments on the cardiovascular function, stress physiology and biochemistry of the sablefish when challenged with hypoxia and elevated temperatures will provide more clarity.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2019.02.004>.

Declarations of interest

None.

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